ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1983, p. 267-272
0066-4804/83/020267-06$02.00/0

Vol. 23, No. 2

Peroxidase-Thiocyanate-Peroxide Antibacterial System Does Not Damage DNA

WILLIAM E. WHITE, JR.,[1]† KENNETH M. PRUITT,[1]* AND BRITTA MANSSON-RAHEMTULLA[2]

Departments of Biochemistry[1] and Pediatric Dentistry, School of Dentistry,[2] University of Alabama in Birmingham, University Station, Birmingham, Alabama 35294

Received 7 September 1982/Accepted 17 November 1982

The hypothiocyanite ion ($OSCN^-$) is a normal component of human saliva. It is a highly reactive oxidizing agent, and at concentrations above the values normally found in human saliva, it inhibits the growth and metabolism of oral bacteria. This finding has led to the suggestion that antibacterial properties of human saliva might be enhanced in vivo by appropriate supplements which elevate $OSCN^-$ concentrations. Since DNA is sensitive to oxidizing agents (hydrogen peroxide attacks nucleosides), high concentrations of $OSCN^-$ in human saliva might damage DNA and produce deleterious effects on the oral mucosa. In the present study, the effect of high $OSCN^-$ concentrations on several mutagen-sensitive *Salmonella typhimurium* strains was determined. These strains are used to detect base-pair substitutions and frameshift mutations. We also studied the effects of $OSCN^-$ on a *Saccharomyces cerevisiae* (yeast) strain commonly employed as a test cell for evaluating the potential of a compound to produce gene conversion, mitotic crossing-over, or reverse mutation. By recording the UV spectra of mixtures of calf thymus DNA and $OSCN^-$, we explored the possible in vitro reactions of this oxidizing agent with eucaryotic genetic material. Our results show that, at concentrations above 10 μM, $OSCN^-$ is toxic for the tested *Salmonella typhimurium* strains. The mutant strains with defects in cell wall lipopolysaccharides are killed more readily by $OSCN^-$ than is the strain lacking these defects. However, $OSCN^-$ was not mutagenic for any of the tested strains. *Saccharomyces cerevisiae* was not affected by $OSCN^-$ even at concentrations above 800 μM. Calf thymus DNA was not oxidized by $OSCN^-$. We conclude that the elevated concentrations of $OSCN^-$ required to produce antibacterial effects in the human mouth pose no threat to the genetic material of host tissues.

The hypothiocyanite ion ($OSCN^-$) is one of the principal oxidized forms of thiocyanate (SCN^-) generated by the peroxidase-SCN^--H_2O_2 system at neutral pH (20). The concentration of this antibacterial factor in milk (12) and saliva (17) can be significantly increased by the addition of SCN^- and H_2O_2. These results have suggested that the antibacterial properties of the peroxidase system might be enhanced in vivo by appropriate supplements (11, 12, 17).

It is important to determine that measures which elevate $OSCN^-$ concentrations above the normal levels (11, 17, 20) found in human secretions do not produce negative side effects. Although there have been no reports of untoward effects of elevated $OSCN^-$ concentrations in human secretions, $OSCN^-$ is a very reactive oxidizing agent (9, 18), and the possibility of its interaction with nucleic acids and of resulting subsequent mutagenic effects should be evaluated.

In recent years, the techniques developed by Ames and co-workers (1) have been used extensively to test compounds for mutagenicity. When Ames et al. tested known carcinogens in their system, they found a correlation of about 90% between the carcinogenicity of tested substances and the capacity of these substances to produce back-mutation in the test strains of *Salmonella* (8). The failure of a test substance to produce mutagenesis in the Ames assay is a strong, although not absolute, indication that the substance is non-carcinogenic. We describe here experiments in which we evaluated bacteriostatic, bactericidal, and mutagenic effects of $OSCN^-$ against three strains of histidine-deficient *Salmonella typhimurium* mutants. We also prepared $OSCN^-$ by nonenzymatic methods and compared the results obtained with those obtained by methods in which peroxidase is

† Present address: Toxicology Branch, Chemical Systems Laboratory, Aberdeen Proving Ground, Aberdeen, MD 21010.

used as a catalyst, and we studied the correlation between antibacterial effects and cell wall properties in the tested strains.

In other experiments, we examined the effect of $OSCN^-$ on a *Saccharomyces cerevisiae* (yeast) strain. Because the cell envelope of *Saccharomyces cerevisiae* has been shown to be permeable to SCN^- (2), we reasoned that it might also be permeable to $OSCN^-$ and that a study of the effects of $OSCN^-$ on this microorganism would provide additional, although indirect, evidence of possible interaction of $OSCN^-$ with genetic material.

Finally, we studied the in vitro reaction of a preparation of $OSCN^-$ with calf thymus DNA. Our thinking was that these experiments would provide direct evidence for possible reactions of $OSCN^-$ with eucaryotic genetic material.

MATERIALS AND METHODS

Bacterial and yeast strains. All *Salmonella typhimurium* strains were obtained from B. N. Ames, University of California, Berkeley. Strain TA1535 contains the *hisG-46* histidine mutation which is used for detecting base-pair substitutions. Strains TA1537 and TA1538 contain the *hisC-3076* and *hisD-3052* mutations, respectively, and are used to detect frameshift mutations (1). All three strains have defects in the *uvrB* gene which render them incapable of excision repair and in the *rfa* mutations which contribute the deep rough character to the cell wall. This property increases the permeability of the cell envelope. In contrast, *hisG-46* (the parent strain of TA1535) has a normal cell wall but is repair deficient.

Saccharomyces cerevisiae D-7 was obtained from Friedrich K. Zimmerman, Tech. Hoch., Darmstadt, W. Germany. This diploid strain contains *trp5-12/trp5-27, ade2-40/ade2-119*, and *ilv1-92/ilv1-92*, which are used to detect gene conversion, mitotic crossing-over, and reverse mutation, respectively (22).

Preparation of $OSCN^-$. $OSCN^-$ was prepared enzymatically from solutions containing potassium SCN (5 mM), bovine lactoperoxidase (2.5 μg/ml; Sigma Chemical Co., St. Louis, Mo.), and hydrogen peroxide (H_2O_2) (2.5 mM) in phosphate-buffered saline (0.01 M, pH 6.5).

The non-enzymatically prepared $OSCN^-$ was extracted from CCl_4 solutions of thiocyanogen $(SCN)_2$ as follows. Silver SCN was synthesized by adding 20.6 g of silver nitrate ($AgNO_3$) in 100 ml of quartz-distilled water to a solution of sodium thiocyanate, also in quartz-distilled water (9.81 g in 100 ml). The precipitate was collected by filtration and washed in cold water. Silver SCN was dried in a vacuum over calcium chloride ($CaCl_2$). The $(SCN)_2$ was prepared by adding dropwise 0.25 g of bromine (Br_2) in 25 ml of carbon tetrachloride (CCl_4) to 3.5 g of silver SCN in 15 ml of CCl_4 (5, 15). The $(SCN)_2$ solution was separated by centrifugation and stored in the dark and cold until use. The concentration of $(SCN)_2$ was determined spectrophotometrically (4). The $OSCN^-$ was prepared immediately before the start of the experiment by extraction of $(SCN)_2$ in sodium hydroxide (NaOH). Two milliliters of the $(SCN)_2$ preparation in 50 ml of CCl_4 were added dropwise into rapidly stirred, ice-cold 0.03 M NaOH (18). $OSCN^-$ was assayed as described by Aune and Thomas (3).

To be able to attach significance to the results, it was necessary to study the stability of $OSCN^-$ in molten agar under the conditions of the bacterial tests. The disappearance of $OSCN^-$ was measured in agar at 45°C under test conditions but with the bacteria omitted. For comparative purposes, the kinetics of disappearance were also studied at 45°C in water and in phosphate-buffered saline. An additional analysis of decay kinetics at 0°C was carried out to estimate an apparent activation energy for the decay process.

Bacteria studies. Because the $OSCN^-$ reacted with some component in nutrient broth, bacteria for mutagenicity testing were grown in a minimal synthetic medium. The original bacteria stock was grown in nutrient broth (Difco Laboratories, Detroit, Mich.) and stored (8% dimethyl sulfoxide) at −70°C in small vials (1). On the morning of the tests, vials were thawed, and 0.2 to 0.3 ml was added to ~15 ml of Vogel-Bonner (21) salts with 2% glucose and supplemented with 10^{-3} M histidine and 10^{-4} M biotin. The bacteria were allowed to grow in a water bath shaker (37°C) for 6 h until the absorbancy at 660 nm was 0.7 to 0.8. Top agar (0.5% agar–0.45% NaCl–10^{-5} M histidine–10^{-5} M biotin) was melted in boiling water and then cooled and maintained at 45°C in a water bath. Bacteria were added (an aliquot of 5% of the agar volume), the bacterial agar suspension (16 ml) was pipetted into 25-ml Erlenmeyer flasks, and selected concentrations of $OSCN^-$ were added. The agar suspension was shaken at 45°C in the same water bath.

Toxicity studies. Five minutes after addition of the $OSCN^-$, 1 ml of agar suspension was diluted to 10 ml with Vogel-Bonner salts. This sample was serially diluted in Vogel-Bonner salts, and 0.1-ml samples were added to 2 ml of molten agar containing 10^{-3} M histidine and 10^{-5} M biotin at 45°C in a melting block apparatus (Fisher Scientific Co., Philadelphia, Pa.). These mixtures were then shaken and poured into bottom agar plates (Vogel-Bonner salts, 2% glucose, 1.5% agar). The 10^{-3} M histidine provided sufficient histidine for all bacteria to grow. Quadruplicate platings were done for each chosen dilution.

Mutagenicity studies. Two-milliliter samples of the agar suspension were pipetted onto bottom agar plates. To confirm that the assay was working at each concentration of $OSCN^-$ tested, three 2-ml samples of the agar suspension were added separately to tubes at 45°C containing 0.2 ml of known mutagens (NaN_3 for strain TA1535, 9-NH_2-acridine for strain TA1537, and 2-NO_2-fluorene for strain TA1538). The 10^{-5} M histidine was sufficient for a few divisions so that the bacteria could produce a translucent lawn.

Controls. Controls were treated in the same way, except no $OSCN^-$ was added. The presence of the deep rough membrane character was confirmed with a drop of crystal violet added to the center of a plate. Because of the cell envelope defect in the deep rough strains, a clear killing zone develops around the point of addition of the crystal violet.

Radial diffusion mutation tests. A 0.1-ml sample of bacteria in 2 ml of top agar was poured onto the plates. After the agar had gelled (5 min), a 4-mm well was cut into the agar so that 0.05 ml of test compound at a high concentration could be accommodated. The compound diffuses radially in such a way that its concen-

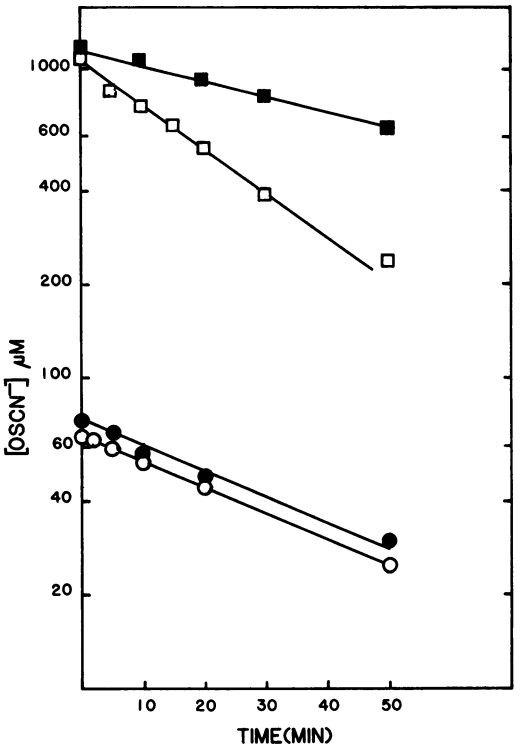

FIG. 1. Decay kinetics of $OSCN^-$. Solutions of $OSCN^-$ were prepared with the bovine lactoperoxidase catalyzed oxidation of SCN^-. Reactions were carried out by adding $OSCN^-$ at t = 0 to phosphate-buffered saline (■, 0°C; □, 45°C), to water (●, 45°C), and to molten agar (○, 45°C). The total $OSCN^-$ was determined as described in the text. Data were analyzed by the method of least squares applied to plots of natural log versus time. From these analyses, the following apparent first-order rate constants (per minute) were determined: ■ = 0.0117, □ = 0.0299, ● = 0.0177, ○ = 0.0193.

tration decreases approximately with the square of the distance. Initially, the high dose is toxic, as indicated by the absence of a bacterial lawn. If the compound is mutagenic, the optimum concentration will be achieved somewhere on the plate and mutants will appear in a ring around the well, in contrast to spontaneous mutants, which are randomly distributed. Farther from the well, the concentration is so low that only spontaneous mutants appear.

Saccharomyces cerevisiae. *Saccharomyces cerevisiae* D-7 was grown from a single colony in a water bath shaken at 30°C in yeast-peptone-dextrose broth containing 1% yeast extract (Difco), 2% Bacto-Peptone (Difco), and 1% glucose to a concentration of 3×10^5/ml as determined by plating. At this point, the colonies were still in log phase. The organisms were centrifuged, washed, suspended in water, and incubated for 1 h with $OSCN^-$ at a cell density of 4×10^6 ml^{-1} at 30°C. The organisms were diluted 10^3 in water and plated on yeast-peptone-dextrose plates containing 1.5% agar. The plates were incubated for 2 days at 30°C and then counted.

Reaction of calf thymus DNA with $OSCN^-$. The DNA (calf thymus DNA, sodium salt, type I; Sigma) was dissolved in water and mixed with enzymatically prepared $OSCN^-$. Two types of experiments were carried out. Oxidation of DNA was monitored by recording the absorbancy at 257 nm (7) for 30 min after addition of $OSCN^-$. The initial concentration of $OSCN^-$ in the reaction mixture was 89 μM. The DNA concentration was sufficient to give an absorbancy at 257 nm of 0.69. Assuming a nucleotide molar extinction coefficient of 6,400 M^{-1} cm^{-1} (6), the calculated concentration is 106 μM in nucleotide residues. In the second series of experiments, the disappearance of $OSCN^-$ was monitored by recording the absorbancy at 233 nm (9) for 20 min after addition of $OSCN^-$. Initial concentrations were 93 μM $OSCN^-$ and 8 μM DNA (nucleotide residues).

RESULTS

Stability of $OSCN^-$. The decomposition of enzymatically generated (see Materials and Methods) $OSCN^-$ was followed at 45 and 0°C. The decomposition followed first-order kinetics at both temperatures (Fig. 1) and was much

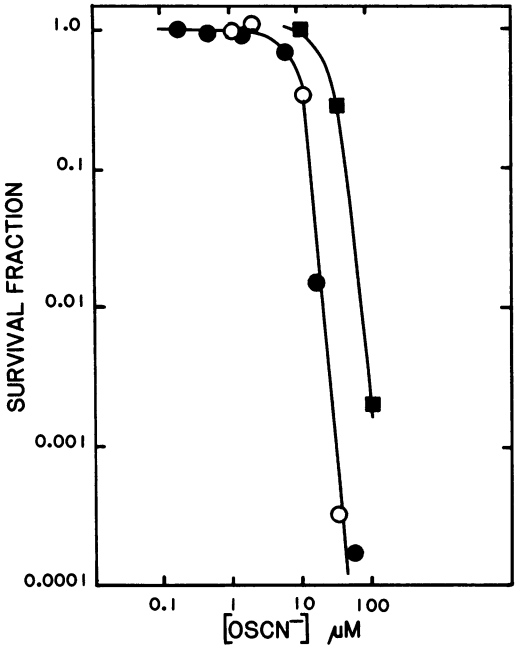

FIG. 2. Effect of $OSCN^-$ concentration on viability of selected strains of *S. typhimurium*. The strains were incubated with the indicated initial concentrations of $OSCN^-$ for 5 min in molten (45°C) agar and then immediately diluted into growth medium for subsequent viability determinations, using agar plates as described in the text. Data are plotted for the parent strain, G-46 (■), and for strain TA1535 with cell envelope defect (○, ●). For this latter strain, $OSCN^-$ prepared both from the purified lactoperoxidase system (●) and from the hydrolysis of $(SCN)_2$ (○) were used. The survival fraction is the ratio of the counts observed for the incubations with $OSCN^-$ to the counts observed for appropriate controls.

TABLE 1. Effect of $OSCN^-$ on mutations in radial diffusion experiments

Strain	No. of mutant colonies obtained with following test substance[a]:		
	Negative control[b]	$OSCN^-$[c] (killing zone [mm])	Positive control (killing zone [mm])[d]
TA1535	66 ± 16	61 ± 10: (2)	>5,000 (0)
TA1537	26 ± 9	26 ± 3: (2)	>2,000 (8)
TA1538	55 ± 3	55 ± 5: (2)	>1,000 (2)

[a] Test strains were plated in top agar immediately before the addition of 0.05 ml of the test substance to the test well (diameter, 4 mm). Numbers ± standard deviations are the number of subsequently observed mutant colonies ($n = 4$).

[b] Dimethyl sulfoxide; nonmutagenic control for random mutations.

[c] Initial concentration, 970 μM.

[d] The following known mutagens were used: sodium azide (initial concentration, 0.02 M; >5,000 colonies), 9-aminoacridine (initial concentration, 0.01 M; >2,000 colonies), 2-nitrofluorene (initial concentration, 0.1 mM; >1,000 colonies).

more rapid at 45°C (half-life, 23 min) than at 0°C (half-life, 59 min).

Bactericidal properties of $OSCN^-$. Both the tested strains of bacteria were killed by $OSCN^-$ (Fig. 2). The same effects were observed with enzymatically prepared $OSCN^-$ and with solutions prepared in the absence of the enzyme. The strain with intact cell wall was more resistant to killing than the defective cell wall mutant. Under the conditions of the mutagenicity test, killing zones of 2 mm were observed for strains TA1535, TA1538, and TA1537 in the diffusion experiments.

Mutagenic properties of $OSCN^-$. Table 1 illustrates the radial diffusion technique and shows that, when the test strains were incubated with known mutagens, more than 1,000 back-mutations were observed in every case. These mutants formed a ring around the well. However, $OSCN^-$ produced only background mutations which were randomly distributed on the plates.

Table 2 shows the absence of mutations in which the concentration of $OSCN^-$ ranged over 4 orders of magnitude. In this study, all bacteria were exposed to the indicated $OSCN^-$ concentration before plating. At the lower doses, there was no killing; at the higher doses, only 1 cell in 10^4 survived. The positive controls indicated that $OSCN^-$ had no effect on mutagenicity until the toxic level was reached. The differences in cell wall permeability are illustrated by comparing the cell wall-defective strain TA1535 (5,000 mutants) with the intact *hisG-46* (100 mutants) parent.

Effect of $OSCN^-$ on *Saccharomyces cerevisiae*. $OSCN^-$ was not toxic for *Saccharomyces cerevisiae* D-7 even at concentrations 10-fold higher than those which killed 99.99% of *Salmonella typhimurium* (Table 3).

Reaction of $OSCN^-$ with calf thymus DNA. Mixtures of the DNA and $OSCN^-$ (generated with the lactoperoxidase-SCN^--H_2O_2 system) did not show any change in their UV spectra over periods of 20 to 30 min at room temperature (21 to 23°C). Oxidation of DNA would have produced significant changes in the UV extinction of nucleoside residues (7), and the reaction of $OSCN^-$ would also have produced a decrease in UV absorbance (9). The lack of change in the continuously recorded absorbance (at 257 and 233 nm) of these reaction mixtures indicated that no significant reactions occurred between nucleosides and $OSCN^-$.

TABLE 2. Effect of incubation of *Salmonella* testor strains with various concentrations of $OSCN^-$

$OSCN^-$ concn (μM)	Mean (± SD) no. of colonies of strain[a]:							
	TA1535		TA1537		TA1538		*hisG-46*	
	Test	Positive control	Test	Positive control	Test	Positive control	Test	Positive control
0	23 ± 5	>5,000	20 ± 3	>2,000	60 ± 6	1,250	2	131 ± 35
0.11	ND		ND		61 ± 6	>1,250	ND	
0.33	22 ± 4	>5,000	21 ± 1	>2,000	52 ±	>1,250	6	89 ± 23
1.1	23 ± 6	>5,000	22 ± 10	>2,000	70 ± 16	>1,250	19 ± 4	187 ± 27
3.3	37 ± 10	>5,000	24 ± 4	>2,000	48 ± 4	>1,250	15 ± 4	73 ± 34
11	29 ± 2	>5,000	32 ± 12	>2,000	55 ± 3	>1,250	10 ± 4	95 ± 48
33	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	15 ± 3	ND
90	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic

[a] Bacteria were incubated for 5 min with the indicated $OSCN^-$ concentration at 45°C in molten top agar before plating. After plating, the agar was allowed to harden at room temperature for 15 min, plates were incubated at 37°C for 2 days, and then the number of mutant colonies were counted. Average number of colonies (± standard deviation) for four plates are given. Positive controls were added immediately before plating and consisted of 1 μmol of NaN_3 per plate for TA1535, 0.1 μmol of 9-aminoacridine per plate for TA1537, 6 nmol of 2-nitrofluorene per plate for TA1538, and 2 μmol of NaN_3 per plate for *hisG-46*. ND, Not determined.

TABLE 3. Survival of *Saccharomyces cerevisiae* exposed to $OSCN^-$[a]

$OSCN^-$ (μM)	No. (mean ± SD) of CFU (n = 4)
0	375 ± 27
100	380 ± 19
215	397 ± 18
430	359 ± 28
860	381 ± 20

[a] Cells of *Saccharomyces cerevisiae* D-7 (4×10^6 ml^{-1}) were incubated with the indicated $OSCN^-$ concentration for 1 h at 30°C, subsequently plated on agar, incubated for 2 days at 29°C, and then counted.

DISCUSSION

In these experiments, the *Salmonella typhimurium* test strains were exposed initially to $OSCN^-$ solutions by incubating reaction mixtures for 5 min at 45°C in molten agar. In the absence of bacteria, these reaction conditions per se did not result in significant decomposition of $OSCN^-$ over the 5-min incubation period. The apparent first-order rate constant for the decomposition of $OSCN^-$ under these conditions was 0.0193 min^{-1} (Fig. 1). Thus, the $OSCN^-$ concentrations to which the bacteria were exposed in the toxicity and mutagenicity tests ranged from 90 to 100% of the initial value.

Apparent first-order kinetics for $OSCN^-$ decomposition were also observed by Thomas (18). The results shown in Fig. 1 are in qualitative agreement with his findings. Decomposition rates were similar in agar and in water at 45°C but were significantly greater in phosphate-buffered saline at this temperature. Thomas (18) also found increased decomposition rates in phosphate solutions. The apparent first-order rate constants for the decomposition in phosphate-buffered saline at 0 and 45°C (Fig. 1) yield a calculated activation energy of 3,600 cal/mol.

There have been few studies of the effects of the lactoperoxidase-SCN^--H_2O_2 system on *Salmonella typhimurium*. Reiter et al. (13) found that a strain of *Salmonella typhimurium* was killed by the system, but the effect required extended incubation periods. Using their data, we estimated a survival fraction (ratio of observed viable counts to initial viable counts) of 0.01 after 2 h of incubation. These authors did not report the temperature of their incubation mixture nor the $OSCN^-$ concentrations which were used. However, the latter could not have exceeded 150 μM under the conditions of their experiments. The 5-min incubation period employed in the present studies gave a survival fraction of 0.01 for all of the tested strains when the initial $OSCN^-$ concentration was in the range of 20 to 100 μM (Fig. 2).

Reiter et al. (13) suggested that the cell envelope structure of *Salmonella typhimurium* exerts a significant influence on the susceptibility of the organism to killing by the lactoperoxidase-SCN^--H_2O_2 system. This suggestion was based on the observations of Tagesson and Stendahl (16) that *Salmonella typhimurium* mutants with cell wall polysaccharide deficiencies were much more susceptible to killing by the myeloperoxidase-I^--H_2O_2 system than were strains with intact cell walls. The results shown in Fig. 2 confirm the relationship between cell envelope structure and peroxidase susceptibility of *Salmonella typhimurium*. Significantly higher $OSCN^-$ concentrations were required to kill the parent (G-46) strain with intact cell wall as compared with the strain (TA1535) with lipopolysaccharide deficiencies. The relationship is also confirmed by the correlation between the toxicity levels observed in the mutagenicity experiments and the degree of cell envelope permeability of the test (Table 2). The toxic concentration of $OSCN^-$ was threefold higher for the strain with intact cell envelope as compared with the mutant strains. This relationship between cell envelope permeability and susceptibility to killing by the peroxidase system may be a general property of gram-negative organisms, since it has also been observed for several different strains of *Escherichia coli* (13, 14, 19).

In addition to $OSCN^-$, the peroxidase-SCN^--H_2O_2 system also generates highly reactive, short-lived intermediates (9, 10). These intermediates have even greater antibacterial effects than does $OSCN^-$ (17). To evaluate the possible contribution of these intermediates to our results, we also prepared $OSCN^-$ by the hydrolysis of $(SCN)_2$ (18), a method which does not result in the generation of these intermediates. The effects of $OSCN^-$ on the parent strain (G-46) (Fig. 2) were identical whether the oxidizing agent was generated by the peroxidase system or by the nonenzymatic method.

The primary objective of the present study was to determine whether the lactoperoxidase-SCN^--H_2O_2 system generated DNA-reactive substances. In our studies, we utilized the sensitive mutagenicity testing scheme developed by Ames and co-workers (1). These strains are also deficient in DNA repair mechanisms and are thus especially sensitive to the effects of substances which damage DNA.

$OSCN^-$ did not produce mutagenic effects even when tested at concentrations more than five times greater than the concentrations which inhibit the metabolism of human dental plaque (17; Table 1). The number of mutations produced by $OSCN^-$ did not differ from the background of random mutations for any of the tested strains. This lack of mutagenicity of

$OSCN^-$ is significant because the test, as we carried it out, was very sensitive to the effects of known mutagens.

The Ames testor strains can also be incubated directly with the test substance and the treated bacteria can be analyzed subsequently for any back-mutations produced by the treatment. Known mutagens produced a very high number of mutations in this test (Table 2). However, the number of mutations produced by incubating the test strains with $OSCN^-$ concentrations up to the level of toxicity did not differ significantly from the background number produced by incubations in the absence of $OSCN^-$. These results show a lack of significant effect of $OSCN^-$ on the DNA of test cells.

To obtain additional evidence of the possible interaction of $OSCN^-$ with DNA, we studied a *Saccharomyces cerevisiae* strain whose envelopes are permeable to anions (2). Even the highest $OSCN^-$ concentrations tested had no effect on the subsequently observed viability of this microorganism (Table 3).

Finally, we used the potential interaction of $OSCN^-$ with calf thymus DNA as a direct model for possible reactions with the DNA of mammalian cells. Hydrogen peroxide will oxidize DNA nucleosides, and these ring-opening reactions produce significant changes in the UV extinction of the affected molecules. However, incubation of calf thymus DNA with $OSCN^-$ resulted in no detectable change in the UV spectrum of the DNA. Under these conditions, $OSCN^-$ does not oxidize DNA. $OSCN^-$ itself has a UV absorbance peak which is eliminated by oxidation reactions (9). When we monitored the UV spectrum at the peak for $OSCN^-$ absorbance in mixtures of calf thymus DNA and $OSCN^-$, we found no significant changes.

In summary, we have tested the effect of $OSCN^-$ on several mutagen-sensitive *Salmonella typhimurium* strains, on a *Saccharomyces cerevisiae* strain permeable to anions, and on calf thymus DNA. Reactions of $OSCN^-$ leading to base-pair substitutions, frameshift mutations, gene conversion, mitotic crossing-over, or reverse mutation would be detected with this group of test organisms. All of the results lead to the conclusion that $OSCN^-$, the principal product of peroxidase-SCN^--H_2O_2 system, does not undergo significant reactions with DNA.

LITERATURE CITED

1. **Ames, B. N., J. McCann, and E. Yamasaki.** 1975. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat. Res. 31:347–364.
2. **Arnold, W. N., and J. S. Lacy.** 1977. Permeability of the cell envelope and osmotic behavior in *Saccharomyces cerevisiae*. J. Bacteriol. 131:564–571.
3. **Aune, T. M., and E. L. Thomas.** 1978. Oxidation of protein sulfhydryls by products of peroxidase-catalyzed oxidation of thiocyanate ion. Biochemistry 17:1005–1010.
4. **Bacon, R. G. R., and R. S. Irwin.** 1958. Thiocyanogen chloride. Part II. Some physical properties of its solutions. J. Chem. Soc. 778–784.
5. **Björck, L., and O. Claesson.** 1980. Correlation between concentration of hypothiocyanate and antibacterial effect of the lactoperoxidase system against *Escherichia coli*. J. Dairy Sci. 63:919–922.
6. **Jordon, D. O.** 1960. Structure of nucleic acids, part 3: the size and shape and some other properties of DNA in solution, p. 178–240. *In* The chemistry of nucleic acids. Butterworths & Co., Ltd., London.
7. **Kochetkov, N. K., E. I. Budovskii, E. D. Sverelov, N. A. Sinukova, M. F. Turchinskii, and V. N. Shibaev.** 1972. Reactions involving the cleavage or rearrangement of heterocyclic rings of nucleic acid bases and their derivatives, p. 381–419. *In* L. Todd and D. M. Brown (ed.), Organic chemistry of nucleic acid, part B. Plenum Publishing Corp., London.
8. **McCann, J., E. Choi, E. Yamasaki, and B. N. Ames.** 1975. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. Proc. Natl. Acad. Sci. U.S.A. 72:5135–5139.
9. **Pruitt, K. M., and J. Tenovuo.** 1982. Kinetics of hypothiocyanite production during peroxidase-catalyzed oxidation of thiocyanate. Biochim. Biophys. Acta 704:204–214.
10. **Pruitt, K. M., J. Tenovuo, R. W. Andrews, and T. McKane.** 1982. Lactoperoxidase-catalyzed oxidation of thiocyanate: a polarographic study of the oxidation products. Biochemistry 21:562–567.
11. **Pruitt, K. M., J. Tenovuo, W. Fleming, and M. Adamson.** 1982. Limiting factors for the generation of hypothiocyanite ion, an antimicrobial agent, in human saliva. Caries Res. 16:315–323.
12. **Reiter, B.** 1981. The contributions of milk to resistance to intestinal infection in the newborn, p. 155–195. *In* H. P. Lambert and C. B. S. Wood (ed.), Immunological aspects of infection in the fetus and newborn. Academic Press, Inc., New York.
13. **Reiter, B., V. M. E. Marshall, L. Björck, and C.-G. Rosén.** 1976. Nonspecific bactericidal activity of the lactoperoxidase-thiocyanate-hydrogen peroxide system of milk against *Escherichia coli* and some gram-negative pathogens. Infect. Immun. 13:800–807.
14. **Sips, H. J., and M. N. Hamers.** 1981. Mechanism of the bactericidal action of myeloperoxidase: increased permeability of the *Escherichia coli* cell envelope. Infect. Immun. 31:11–16.
15. **Söderbäck, E.** 1919. Studien über das freie Rhodan. Liebigs Ann. Chem. 419:217–219.
16. **Tagesson, C., and O. Stendahl.** 1973. Influence of the cell surface lipopolysaccharide structure of *Salmonella typhimurium* on resistance to intracellular bactericidal systems. Acta Pathol. Microbiol. Scand. Sect. B 81:473–480.
17. **Tenovuo, J., B. Mansson-Rahemtulla, K. M. Pruitt, and R. Arnold.** 1981. Inhibition of dental plaque acid production by the salivary lactoperoxidase antimicrobial system. Infect. Immun. 34:208–214.
18. **Thomas, E. L.** 1981. Lactoperoxidase-catalyzed oxidation of thiocyanate: the equilibria between oxidized forms of thiocyanate. Biochemistry 20:3273–3280.
19. **Thomas, E. L., and T. M. Aune.** 1978. Susceptibility of *Escherichia coli* to bactericidal action of lactoperoxidase, peroxide and iodide or thiocyanate. Antimicrob. Agents Chemother. 13:261–265.
20. **Thomas, E. L., K. P. Bates, and M. M. Jefferson.** 1980. Hypothiocyanite ion: detection of the antimicrobial agent in human saliva. J. Dent. Res. 59:1466–1472.
21. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. 218:97–106.
22. **Zimmerman, F. K., R. Kern, and H. Rosenberger.** 1975. A yeast strain for simultaneous detection of induced mitotic crossing over, mitotic gene conversion and reverse mutation. Mutat. Res. 28:381–385.